International Journal of Infectious Diseases 17 (2013) e1062–e1063

Contents lists available at SciVerse ScienceDirect

International Journal of Infectious Diseases

journal homepage: www.elsevier.com/locate/ijid

Case Report

Breakthrough bacteremia due to *Clostridium tertium* in a patient with neutropenic fever, and identification by MALDI-TOF mass spectrometry

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ARTICLE INFO

Article history:
Received 4 February 2013
Received in revised form 25 February 2013
Accepted 2 March 2013

Corresponding Editor: Eskild Petersen, Aarhus, Denmark

Keywords:
Bacteremia
Clostridium tertium
Neutropenia
Antibiotic resistance
Mass spectrometry

SUMMARY

Clostridium tertium is rare in a human clinical specimen and its pathogenicity is often uncertain. However, the organism has been increasingly recognized as a cause of bacteremia and other infections in immunocompromised patients, especially those with hematologic malignancies. The diagnosis and treatment of *C. tertium* are difficult due to its growth pattern, micromorphology, and antibiotic resistance. The organism can easily be misidentified as Gram-positive aerobic rods such as *Bacillus* species, usually considered as a contaminant. Furthermore, it is not covered by empirical treatment with many broad-spectrum antibiotics. Here we report a case of breakthrough bacteremia due to *C. tertium* that occurred in a patient with acute leukemia and neutropenic fever, who was treated with an empirical regimen of ceftazidime and amikacin. The bacterium was rapidly identified by new mass spectrometry technology (MALDI-TOF MS) and the patient recovered under meropenem and vancomycin treatment, without complications.

1. Introduction

Clostridium tertium is an endospore-forming Gram-positive bacillus, which may grow aerobically and does not produce exotoxins. It can be isolated in soil and the gastrointestinal tract of humans and other animals, but rarely occurs in clinical samples.[1] To date, its role as a human pathogen remains uncertain.[2] The organism grows easily on conventional media, but misidentification can occur due to its aerotolerance and Gram-variable appearance.[1] In contrast to most other clostridial species, treatment can be a challenge, since resistance to various antibiotics including third- and fourth-generation cephalosporins, seems to be common.[1]

We report a case of breakthrough bacteremia in a patient with neutropenic fever under treatment with a standard broad-spectrum antibiotic regimen.

2. Case report

A 47-year-old woman presented with a history of 5 days of dry cough and sharp left-sided thoracic pain accompanied by fever up to 38 °C, malaise, and headache. Two months previously she had been treated with levofloxacin for maxillary sinusitis; otherwise she reported no relevant previous illnesses. A physical examination was unremarkable except for left upper quadrant abdominal pain on deep palpation. Chest radiography showed no pathological findings. Laboratory evaluation revealed a blood leukocyte count of 78.4×10^9/l with 97% blasts and an absolute neutrophil count of 0.784×10^9 cells/l, platelet count of 12×10^9/l, hemoglobin of 10.2 mg/dl, and C-reactive protein (CRP) of 6.02 mg/dl. Computed tomography scanning of the abdomen and pelvis demonstrated splenomegaly of 14 cm. Based on these test results the patient was hospitalized with the suspicion of acute leukemia. Urine and blood cultures were taken and empiric antibiotic therapy with ceftazidime and amikacin was initiated. Further hematologic and genetic studies confirmed the diagnosis of Philadelphia chromosome-negative common acute lymphoblastic leukemia. Microbiological cultures remained negative, and after 2 days of antibiotic treatment, the patient was afebrile and started chemotherapy with fractionated cyclophosphamide, vincristine, doxorubicin, and dexamethasone (Hyper-CVAD). During the following days she developed progressive neutropenia. Therefore, the antibiotic regimen was maintained and the patient remained afebrile and clinically stable. On day 17 after the start of chemotherapy, the patient presented with a fever up to 40 °C; she was hemodynamically stable and without other symptoms except transient

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1201-9712/$36.00 – see front matter
http://dx.doi.org/10.1016/j.ijid.2013.03.005

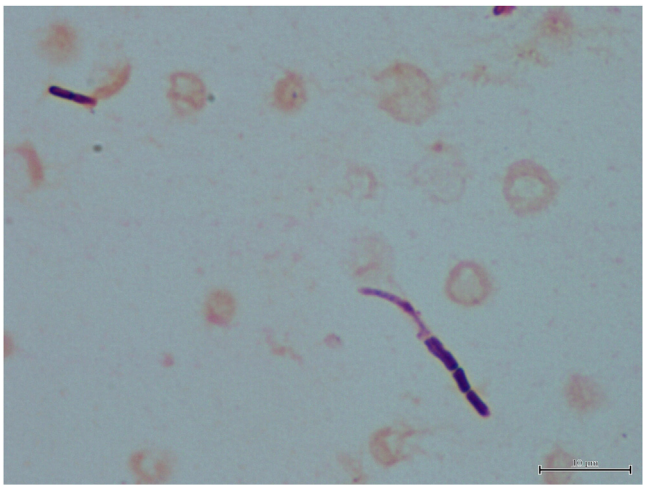

Figure 1. Gram-stain of *Clostridium tertium* in blood culture medium.

abdominal pain. Laboratory tests revealed a leukocyte count of 0.1×10^9/l, platelets of 37×10^9/l, hemoglobin of 9.9 g/dl, and CRP of 17.3 mg/dl. Two sets of blood cultures were drawn and the antimicrobial treatment was changed to meropenem, vancomycin, and voriconazole.

Within 16 to 21 h, all four blood culture media became positive (BacT/Alert 3D system with FAN aerobic bottles; bioMérieux, Marcy l'Etoile, France). Gram staining revealed large Gram-variable bacilli, partly growing in short chains (Figure 1). Overnight aerobic cultivation on 5% sheep blood agar (bioMérieux) at 35 °C showed small, semitransparent, shiny colonies of Gram-variable rods. Examination by mass spectrometry identified the strain as *Clostridium tertium* with a high level of reliability; Microflex LT (Bruker Daltonics, Bremen, Germany) gave an identification log score of 2.517 (above 2.0 is considered reliable for species identification) and Vitek MS (bioMérieux) reported the same result with 99.9% certainty. The following day, this result was confirmed by conventional biochemical methods (VITEK 2 Compact; bioMérieux). The organism also grew under anaerobic conditions in similar but bigger colonies and microscopically with terminal spores. Antibiotic susceptibility testing by agar dilution technique performed at the national reference laboratory (Instituto de Salud Pública, Santiago, Chile) demonstrated sensitivity to metronidazole (minimum inhibitory concentration (MIC) 1.0 μg/ml) and moxifloxacin (MIC 0.25 μg/ml), but resistance to ceftriaxone (MIC >32 μg/ml).

The patient remained clinically stable and became afebrile after 3 days. Treatment with meropenem and vancomycin was maintained until day 12 of the regimen, when the patient, who had a history of penicillin allergy, developed an urticarial rash and meropenem was replaced by amikacin. This antibiotic therapy was continued for a further 2 weeks. The patient recovered from the neutropenia and continued her chemotherapy in the following weeks without recurrence of *C. tertium* bacteremia.

3. Discussion

Clostridium tertium has long been considered apathogenic, since it only rarely occurred in mixed infections of traumatic wounds. In recent years the organism has been isolated in cases of bacteremia, spontaneous bacterial peritonitis, enterocolitis, meningitis, septic arthritis, necrotizing fasciitis, post-traumatic brain abscess, and complicated pneumonia in mono- or polymicrobial infections.[2,3] In cases of clostridial bacteremia, *C. tertium* is the second most frequently isolated species after *Clostridium perfringens*.[1] The main risk factors seem to be intestinal mucosal injury, neutropenia, and exposure to beta-lactam antibiotics.[4] Similar to *Clostridium difficile*, the use of broad-spectrum antibiotics such as third-generation cephalosporins might predispose to intestinal colonization with *C. tertium*. Patients with hematologic malignancies combine several of these risk factors, and cases of *C. tertium* bacteremia in those patients have been described in association with diarrhea, colonic bleeding, and peri-anal cellulitis.[4] However, as our case demonstrates, such distinct intestinal symptoms might also be absent. In cases of neutropenic fever, international guidelines recommend the empirical use of broad-spectrum beta-lactam antibiotics as monotherapy or in combination with other drugs such as aminoglycosides.[5] Empirical treatment for neutropenic fever often does not cover *C. tertium* and our case highlights that breakthrough bacteremia might occur. In these situations, a rapid identification and communication between the microbiologist and clinician is crucial.[1] Because of its micromorphology and growth pattern, *C. tertium* is often mistaken for *Bacillus* or *Lactobacillus* species, both considered as contaminants or apathogenic. Therefore thorough identification of this species is critical. Unfortunately, traditional identification of anaerobes is time-consuming and cumbersome. New methods based on mass spectrometry such as MALDI-TOF MS (matrix-assisted laser desorption/ionization time-of-flight mass spectrometry), which have been developed for the rapid identification of bacterial strains, might offer new possibilities for this group of microorganisms.[6,7] In our case, the two commercial systems that are available at the moment, Microflex LT and Vitek MS, were able to identify *C. tertium* reliably and faster than traditional methods.

The treatment of *C. tertium* infection is complicated due to resistance to various antibiotics, including various beta-lactam antibiotics (such as third- and fourth-generation cephalosporins), clindamycin, daptomycin, and cotrimoxazole.[1–3] Older reports state resistance to metronidazole, but this has not been confirmed in more recent publications. Available data indicate sensitivity to vancomycin, carbapenems, and quinolones. Still, studies examining sufficient numbers of strains are lacking. As in our case, clinical resolution of bacteremia due to *C. tertium* occurs rapidly if adequate treatment is chosen.[4]

Our case highlights that *C. tertium* is able to cause breakthrough bacteremia in patients with neutropenic fever receiving standard empiric antibiotics and that rapid identification of this unusual pathogen can be achieved by new MALDI-TOF technology.

Conflict of interest: All authors have no conflict of interest. This paper was written without any financial support.

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